Peroxisome biogenesis in mammalian cells: the impact of genes and environment

Rebecca L. Farr[a,b], Celien Lismont[a], Stanley R. Terlecky[b], Marc Fransen[a,*]

[a]Laboratory of Lipid Biochemistry and Protein Interactions, Department of Cellular and Molecular Medicine, KU Leuven – University of Leuven, Herestraat 49 box 601, B-3000 Leuven, Belgium
[b]Department of Pharmacology, Wayne State University School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201, USA

***Correspondence:**
Dr. Marc Fransen
KU Leuven – University of Leuven
Department of Cellular and Molecular Medicine
Laboratory of Lipid Biochemistry and Protein Interactions
Herestraat 49 box 601
3000 Leuven, Belgium
Telephone: 00 32 16 330 114
Email: marc.fransen@med.kuleuven.be

Abstract

The initiation and progression of many human diseases are mediated by a complex interplay of genetic, epigenetic, and environmental factors. As all diseases begin with an imbalance at the cellular level, it is essential to understand how various types of molecular aberrations, metabolic changes, and environmental stressors function as switching points in essential communication networks. In recent years, peroxisomes have emerged as important intracellular hubs for redox-, lipid-, inflammatory-, and nucleic acid-mediated signaling pathways. In this review, we focus on how nature and nurture modulate peroxisome biogenesis and function in mammalian cells. First, we review emerging evidence that changes in peroxisome activity can be linked to the epigenetic regulation of cell function. Next, we outline how defects in peroxisome biogenesis may directly impact cellular pathways involved in the development of disease. In addition, we discuss how changes in the cellular microenvironment can modulate peroxisome biogenesis and function. Finally, given the importance of peroxisome function in multiple aspects of health, disease, and aging, we highlight the need for more research in this still understudied field.

Keywords

Age-related disease; biogenesis disorder; epigenetics; intracellular signaling; mitochondria; peroxisome

1. Introduction

Throughout their lifetime, organisms are continually exposed to a multitude of genetic, biological, environmental, and behavioral risk factors governing disease susceptibility. To survive and flourish against such a backdrop, they have to react appropriately to new circumstances as they arise. Most, if not all, organisms have the ability to respond to internal and external stimuli with altered programs of gene expression. This temporally and spatially regulated process, often referred to as 'epigenetic reprogramming', is driven in large part by changes in chromatin structure (e.g., DNA methylation and histone modifications) and gene transcription levels (e.g., transcription factor regulation and RNA processing) [1]. In general, these changes are coordinated by a diverse array of signals related to cellular metabolic state (e.g., NAD^+/NADH ratios, tricarboxylic acid (TCA)[1] cycle intermediates, total and reduced glutathione concentrations, and acetyl-coenzyme A (acetyl-CoA) levels) [2]. Over recent decades, peroxisomes have emerged as key regulators in overall cellular lipid metabolism [3]. In addition, these organelles have been recognized as important intracellular hubs for redox-, lipid-, and inflammatory-mediated signaling pathways, and – very recently – as the primary sites that initiate type III interferon expression in response to viral and bacterial infections (see [4-6], and references therein). Despite these developments, the cause and effect relationships that exist between peroxisomal (dys)functions and epigenetic alterations are just starting to be explored.

In the following sections, we first provide background information on the mechanisms by which cells integrate genetic and environmental stimuli and translate them into phenotypic outcomes. Next, we explore the link between genetic/environmental interactions and peroxisome biogenesis/function in mammalian cells. Finally, we highlight research directions designed to extend our knowledge in these areas. This is of paramount importance, given that peroxisomes play a pivotal role in human physiology and that effective therapeutic strategies for treatment of patients with peroxisomal deficiencies are still very much limited [7].

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[1]Abbreviations: 4-PBA, 4-phenylbutyrate; 5mC, 5-methylcytosine; α-KG, α-ketoglutarate; ABCD, ATP-binding cassette, subfamily D; ART, ADP-ribosyltransferase; CoA, coenzyme A; DDM, DNA demethylase; DHA, docosahexaenoic acid; DNMT, DNA methyltransferase; ERT, enzyme replacement therapy; FAD, flavin adenine dinucleotide; FDA, United States Food and Drug Administration; GSH (reduced) glutathione; GSSG, oxidized glutathione; HAT, histone acetylase; HDAC, histone deacetylase; HDM, histone demethylase; HIF, hypoxia inducible factor; HMT, histone methyltransferase; JmjC, Jumonji C; IDH, isocitrate dehydrogenase; IRD, infantile Refsum disease; miR, microRNA; NAD(P)(H), (reduced) nicotinamide adenine dinucleotide (phosphate); NALD, neonatal adrenoleukodystrophy; ncRNA, non-coding RNA; PARP, poly(ADP-ribose) polymerase; PBD, peroxisome biogenesis disorder; PED, peroxisomal enzyme/transporter deficiency; PPAR, peroxisome proliferator-activated receptor; PPARGC1A, PPAR γ coactivator 1α; PTS1, C-terminal peroxisomal targeting signal; RCDP, rhizomelic chondrodysplasia punctata; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAHA, suberoylanilide hydroxamic acid; SAM, S-adenosylmethionine; RXR, retinoid X receptor; TCA, tricarboxylic acid; VLCFA, very-long-chain fatty acid; X-ALD, X-linked adrenoleukodystrophy; ZS, Zellweger syndrome; ZSD, Zellweger spectrum disorder.

2. Molecular mechanisms of epigenetics

Epigenetic changes are a critically important mechanism by which the environment directly impacts gene expression without changing the underlying genomic sequence. As currently understood, these changes are brought about by DNA methylations, histone modifications, and non-coding RNA regulations [1]. In the following sections, we briefly outline epigenetic alterations that can be directly, or indirectly, linked to changes in peroxisomal function.

2.1. DNA methylations

DNA methylation is a process in which methyl groups are added to cytosine or adenine nucleotides in genomic DNA. In mammals, methylation typically takes place on cytosine residues immediately preceding guanine bases. As cytosine methylation both physically impedes transcription factor binding and attracts specific methyl-DNA binding proteins that subsequently recruit other chromatin remodeling proteins that can modify histones (e.g., histone deacetylases; see 2.2.2.), this process is, in general, associated with transcriptional repression [8]. DNA methylation is catalyzed by a family of S-adenosylmethionine (SAM)-dependent enzymes, called DNA methyltransferases (DNMTs), that transfer a methyl group from SAM to the 5-position of cytosine to form 5-methylcytosine (5mC), yielding S-adenosylhomocysteine (SAH) as a byproduct [9]. DNA demethylation can be achieved in both passive and active fashion. For example, as DNMT1 maintains methylation during DNA replication, its inhibition allows newly incorporated cytosine to remain unmethylated [9]. Active DNA demethylation in mammals is a complex multistep process that is controlled by DNA demethylases (DDMs) that belong to different protein families (e.g., TET, BER, and AID/APOBEC). For additional details regarding these enzymes and the chemical intermediates that are formed during the demethylation process, we refer the reader to Kohli and Zhang [10].

2.2. Histone modifications

Histones are a family of basic proteins that package nuclear DNA into structural units, called nucleosomes. These proteins can undergo a wide variety of posttranslational modifications, including methylation, acetylation, ADP-ribosylation, phosphorylation, ubiquitination, and sumoylation. These and other modifications contribute to a precise regulation of gene expression by controlling chromatin conformation (e.g., through electrostatic and structural changes) and providing binding sites for non-histone DNA-binding proteins such as transcription factors, transcriptional coactivators, and chromatin remodeling complexes [11]. As there is little evidence that links peroxisomes to histone phosphorylation, ubiquitination, and sumoylation, these modifications will not be considered further here.

2.2.1. Histone methylations

In mammals, there are different classes of histone methyltransferases (HMTs) and histone demethylases (HDMs) that control the methylation status of particular lysine and arginine residues in histones. The functional outcome of histone methylation strongly depends on the number of methyl groups that are added and the location/context of where they occur [8]. Like DNMTs, all HMTs use SAM as a cofactor and methyl donor and produce SAH as a byproduct. HDMs are classified into two distinct groups depending on their catalytic mechanism: lysine-specific demethylases, which are FAD-dependent amine oxidases; and demethylases containing

a Jumonji C domain (JmjC) that catalyze a dioxygenase reaction dependent on Fe^{2+} and α-ketoglutarate (α-KG) [12].

2.2.2. Histone acetylations

Histones can be acetylated on specific lysine residues. This process neutralizes the basic charge of these residues, thereby promoting a general relaxation of chromatin structure and an induction of gene expression [8]. The acetylation status of histones is controlled by a dynamic interplay of histone acetylases (HATs) and histone deacetylases (HDACs). HATs require cytoplasmic acetyl-CoA as donor of the acetyl moiety and release free coenzyme A (CoA) [13]. HDACs yield free acetate that subsequently can be incorporated into acetyl-CoA by acetyl-CoA synthetases. HATs and HDACs are classified into multiple protein families, each having distinct opportunities for mechanistic regulation [8]. In the context of this review, it is important to mention that class I and class II HDACs (the non-sirtuin HDACs) share a catalytic mechanism that involves the coordination of a divalent metal ion, and class III HDACs (the sirtuins) are NAD^+-dependent histone deacetylases whose activity is controlled by the cellular [NAD^+]/[NADH] ratio. Note also that the activity of the non-sirtuin HDACs is influenced by the availability of metabolic intermediates such as free CoA, CoA-derivatives, butyrate, and NADPH [14].

2.2.3. Histone ADP-ribosylation

ADP-ribosylation is an important posttranslational modification in which one or more ADP-ribose moieties are added to a protein. The addition of ADP-ribosome units to histones is catalyzed by members of the poly(ADP-ribose) polymerase (PARP) protein family and occurs preferentially on glutamate or lysine residues [8]. This modification can affect gene expression at various levels (e.g., relaxation of chromatin structure, transcription factor binding, and mRNA processing). Importantly, all ADP-ribosyltransferase (ART) family members consume NAD^+ and release nicotinamide as a byproduct [8]; and excessive PARP activity may cause cell dysfunction (or even cell death) due to a depletion of NAD^+ and the subsequent drop in ATP levels [8]. Mono- and poly(ADP-ribose) moieties can also be enzymatically removed from histones - for more details, see [15].

2.2.4. Regulatory non-coding RNAs

The discovery that some classes of non-coding RNAs (ncRNAs) can also silence transcriptional activity has added an additional layer of complexity to how genes are expressed. The modes of action of these regulatory ncRNAs can vary depending on their size, structure and function. For example, long (>200 nucleotides) regulatory ncRNAs, which are often tissue-specifically expressed and involved in the long-term silencing of particular developmental control genes [8], can function as decoys, scaffolds, or guides for regulatory proteins and protein complexes [16]. Other regulatory ncRNAs such as microRNAs (miRs; ~21-22 nucleotides), and piwi-interacting RNAs (~24-30 nucleotides), can negatively impact gene expression by binding to the 3'-untranslated region of target mRNAs [1]. Importantly, regulatory ncRNAs have emerged as critical factors in cellular metabolism and development; altered expression of these molecules has been implicated in a number of diseases [1].

3. Cellular metabolism and epigenetics

As should be clear from the previous section, many of the enzymes involved in epigenetic regulation require intermediates of cellular metabolism to exert their function. This implies that these enzymes can function as metabolic sensors, and that fluctuations in cellular metabolic state (e.g., in response to normal or pathological stimuli) are likely to lead to changes in gene expression [17-21]. Examples of metabolites involved in such sensing mechanisms are listed in Table 1. Importantly, the regulation of epigenetic processes through metabolites involves multiple layers of complexity. For example, as members of the sirtuin and PARP families exert opposite effects on chromatin structure and gene regulation, a limited availability of NAD^+ may lead to a direct competition between these different types of chromatin-altering enzymes. In addition, as $NAD(P)^+$, FAD, and glutathione (or γ-glutamyl-cysteinyl-glycine, a thiol-containing tripeptide that can exist in a reduced (GSH) or oxidized (GSSG) state) play an important role in maintenance of the cellular redox state, it is clear that epigenetic modifications are also affected by to oxidative stress. In this context, it is relevant to note that (i) redox changes can directly regulate HDAC activities, (ii) the activities of JmjC-HDMs and TET 5mC hydroxylases are controlled by oxygen tension, and (iii) Dicer, a central enzyme in miR processing, functions as an oxidative stress-responsive protein (see [8], and references therein). Epigenetic regulators may also employ different mechanisms for translating metabolic differences into distinct gene expression patterns. On one hand, they may directly modify genomic DNA and histones to regulate chromatin condensation. On the other hand, they may posttranslationally modify transcriptional regulators. Finally, it is important to note that epigenetic modifications also play a central role in the regulation of lipid and other metabolic pathways [17].

To regenerate their pool of GSH, cells have to either recycle GSSG to GSH with the help of an NADPH-dependent glutathione reductase (GR) or synthesize new GSH. The latter pathway is especially important under conditions of prolonged exposure to oxidative stress, in which NADPH stores become depleted and cells may lose GSH due to export of GSSG and GSH-adducts [8]. However, as (i) the rate-limiting substrate for *de novo* GSH biosynthesis is cysteine; (ii) the formation of this amino acid from methionine occurs via a series of reactions involving, among other intermediates, SAM and SAH; and (iii) SAM functions as a universal methyl donor for methyltransferases - *de novo* synthesis of GSH may very well have a major impact on epigenetic status [8,22].

4. Peroxisomes and epigenetics

Peroxisomes harbor a complex set of enzymes that participate in diverse metabolic pathways, such as α- and β-oxidation of fatty acids, detoxification of ROS, and biosynthesis of docosahexaenoic acid, bile acids, and ether-phospholipids [3,23,24]. As these processes generate a variety of intermediates and by-products that may potentially serve as substrates or inhibitors of multiple epigenetic regulators, it is very likely that these organelles participate in epigenetic signaling (Fig. 1). However, despite this potential connection, the precise role of peroxisomes in epigenetic programming remains a largely uncharted field. In the next sections, we outline how peroxisomal lipid and redox metabolism can potentially influence the levels of key metabolites that are important for a number of transcriptional and epigenetic processes. Then, we review how peroxisome (dys)function may influence the epigenetic landscape.

4.1. Linking peroxisomal metabolism and epigenetics

4.1.1. Peroxisomal lipid metabolism

A major function of peroxisomes is the breakdown of a select set of lipophilic carboxylic acids through α- and β-oxidation pathways [3]. These processes involve a set of cosubstrates (e.g., FAD, $NAD(P)^+$, O_2, α-KG) and metabolites (e.g., acetyl-CoA, succinate) that can potentially regulate the activity of epigenetic modifiers. For example, the first step (acetyl-CoA dehydrogenation) of peroxisomal β-oxidation requires FAD-dependent oxidases that pass electrons to O_2, thereby yielding H_2O_2; the second (2-enoyl-CoA hydration) and third (3-hydroxyacyl-CoA dehydrogenation) steps of this process are catalyzed by NAD^+-dependent multifunctional proteins; and the final step (3-ketoacyl-CoA thiolysis), which is catalyzed by either acetyl-CoA acyltransferase or sterol-carrier-protein X (SCPx), results in the formation of acetyl-CoA or propionyl-CoA [3]. Given that peroxisomes also contain a highly specific succinyl-CoA thioesterase, β-oxidation of dicarboxylic acids may lead to the formation of succinate [25]. Note that succinate can also be produced by phytanoyl-CoA α-hydroxylase, a peroxisomal α-oxidation enzyme that functions as a Fe^{2+}-, α-KG-, and O_2-dependent dioxygenase that hydroxylates phytanoyl-CoA at position 2 (thereby converting α-KG into CO_2 and succinate) [3].

Importantly, as epigenetic modifications are strongly influenced by metabolite availability, a *conditio sine qua non* to link peroxisomal lipid metabolism to such events is that relevant cosubstrates (e.g., $NAD(P)^+$/NAD(P)H, α-KG) and metabolites (e.g., acetate/acetyl-CoA, succinate) can cross the peroxisomal membrane. As peroxisomes lack a citric acid cycle and respiratory chain, continued β-oxidation requires free CoA, the reoxidation of NADH, the regeneration of NADPH, and the export of shortened substrates [24]. Most β-oxidation substrates are activated outside peroxisomes, and their CoA forms are subsequently translocated across the organelle membrane through ATP-binding cassette transporters of the D subfamily (ABCD) [3]. The shortened substrates (e.g., acetyl-CoA, propionyl-CoA, succinyl-CoA, and other acyl-CoAs) are enzymatically converted to their free acids (e.g., by acetyl-CoA thioesterases) or carnitine esters (e.g., by acyl-carnitine transferases), thereby releasing CoA inside peroxisomes [3]. This process not only prevents CoA trapping, but also provides an acyl-CoA thioesterase-dependent feedback mechanism to regulate the CoA and acetyl-CoA levels inside the organelle [25]. The free acids and carnitine esters can then be used for fatty acid elongation or leave the organelle by transporters (e.g., OCTN3) or by passive diffusion through channels (e.g., PXMP2) [26]. CoA can be reused by peroxisomal thiolases or acetyl-CoA synthetases [27], or degraded by the peroxisomal Nudix hydrolases NUDT7 and NUDT19 (see [28], and references therein). In general, the peroxisomal membrane is considered to be impermeable to $NAD(P)^+$ and NAD(P)H. The NADH produced inside peroxisomes is thought to reoxidize to NAD^+ through lactate/pyruvate and glycerophosphate redox shuttles [26]. The intraperoxisomal reduction of $NADP^+$ to NADPH is catalyzed by isocitrate dehydrogenase 1 (IDH1) and glucose-6-phoshate dehydrogenase, two enzymes that are located in multiple subcellular compartments [26]. The peroxisomal levels of $NAD(P)^+$ and NAD(P)H are controlled by NUDT12, a peroxisomal Nudix NADH-diphosphatase [28], and SLC25A17 - a peroxisomal membrane protein that belongs to the family of mitochondrial solute carriers [29]. Note that the latter protein is not only thought to function as an NAD^+ transporter, but also as a transporter of free CoA and FAD [29]. Finally, given the properties and exclusion limit (~0.6 kDa) of the channel-forming protein PXMP2, small metabolic intermediates such as acetate, propionate, succinate, and α-KG can be expected to freely diffuse across the peroxisomal membrane [26].

However, note that the existence of a α-KG/isocitrate transporter in the peroxisomal membrane has been reported [30].

4.1.2. Peroxisomal redox metabolism

Over the years, it has become apparent that peroxisomes are central players in cellular redox metabolism [5]. They contain different sets of ROS-producing enzymes, of which the H_2O_2-generating FAD-linked oxidases (see 4.1.1.) represent the largest group. Also, the organelles are well equipped with a variety of antioxidant defense mechanisms, of which the H_2O_2-degrading enzyme catalase is perhaps the best characterized. So far, no consensus has emerged whether or not peroxisomes function as net sources, or sinks, of ROS. This most likely depends on the growth environment and (patho)physiological state of the cell. For example, an increase in peroxisomal fatty acid metabolism leads to an increase in peroxisomal H_2O_2 production (see [5], and references therein). The H_2O_2 generated in this process may be disposed by catalase or leak out into the cytosol [31]. Importantly, alterations in peroxisomal H_2O_2 metabolism have been shown to influence cellular GSH/GSSG balance and protein disulfide content [5]. Note that, as (i) NADPH is required for the GR-catalyzed regeneration of GSH from GSSG (see 3.3.), and (ii) decreased NADPH/$NADP^+$ ratios also affect NADH/NAD^+ ratios due to the modulating capacity of NAD^+ kinases on the equilibrium between NADPH/$NADP^+$ and NADH/NAD^+ ratios [20], a decrease in the GSH/GSSG ratio will rapidly increase the NAD^+/NADH ratio. Like other small metabolites, GSH may freely penetrate the peroxisomal membrane through PXMP2 [26]. How GSSG is reduced in or exported out of the peroxisomal matrix, remains to be investigated. Finally, it is important to emphasize that peroxisome-derived ROS may influence epigenetic signaling through mechanisms other than affecting the levels of metabolites that control the activity of chromatin modifiers. Indeed, previous studies have revealed that changes in peroxisomal H_2O_2 metabolism can directly or indirectly modulate the activity of various transcription factors (e.g., NF-κB, CREB1, and PPARGC1A) (see [5], and references therein). In addition, as (i) peroxisomal and mitochondrial metabolism are closely intertwined [5], and (ii) alterations in mitochondrial metabolism may heavily influence the epigenome [32] - it is very likely that changes in peroxisome activity also lead to epigenetic remodeling through alterations in mitochondrial function.

4.2. Peroxisome (dys)function and epigenetics: a fairly unexplored twist

As is evident from the material presented above, peroxisomal metabolism can be linked to metabolites that play a crucial role in epigenetic processes (Fig. 1). However, direct evidence that peroxisomes do indeed play a role in epigenetic programming under normal physiological conditions is lacking. Therefore, in the following sections, we focus on the (aberrant) epigenetic events that arise as a consequence of peroxisomal dysfunction. For clarity, we first provide background information about how defects in peroxisome biogenesis and function may impact human physiology.

4.2.1. Compromised peroxisome function and disease

Peroxisome biogenesis is mediated by proteins called peroxins – molecules localized variously to the organelle and cytosol. When these essential molecules are absent or non-functional, havoc can reign on the organelle, on the cell, and on the organism. Complex biochemical pathways are compromised, resulting in such metabolic errors as an accumulation of potentially toxic unprocessed fatty acids and an inability to synthesize essential docosahexaenoic acid, bile acids, and plasmalogens - among other

molecules. Typically, these alterations of metabolism manifest themselves in devastating phenotypes, with many human patients succumbing to the disease before age two [7]. The peripheral nervous system is often impacted, as are the brain, liver, kidneys, and eyes. These pathologies often translate into hypotonia, seizures, gliosis, hepatomegaly, neuronal demyelination, sensory deficits, and developmental delays [7].

At the molecular level, peroxisomal diseases can be classified into two groups: peroxisome biogenesis disorders (PBDs) and single enzyme/transporter deficiencies (PEDs). The former occur when an autosomal recessive mutation exists in one or more *PEX* genes (encoding peroxins). Mutations in 14 of the 16 *PEX* genes result in a PBD; there are no known diseases associated with PEX11α or PEX11γ mutations [7]. However, recent findings suggest that a PEX11α deficiency in mice is associated with aggravated interstitial renal lesions [33]. The PBDs encompass the Zellweger spectrum disorders (ZSDs) and rhizomelic chondrodysplasia punctata (RCDP) type 1. The ZSDs include, in order of decreasing clinical severity, Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD). So far, no genotype-phenotype correlation has been found, and the phenotypic severity of this group of disorders is mainly determined by the residual activity of the mutated peroxin. RCDP type 1 is caused by mutations in *PEX7*, which encodes the receptor for proteins having an N-terminal peroxisomal targeting signal (PTS2). As hubs of cellular metabolism, peroxisomes contain more than 60 distinct enzyme constituents and metabolite transporters [34]. This alone sets the stage for many PEDs, which occur when one of these critical enzymes is dysfunctional or absent. These can be subclassified into different categories according to the peroxisomal function that is actually lost (e.g., fatty acid α-oxidation, fatty acid β-oxidation, ether-phospholipid biosynthesis, glyoxylate metabolism, or H_2O_2 metabolism) [35]. The severity of the disease depends on the enzyme involved, with a broad range of clinical manifestations possible [35].

Even when the peroxisomes assemble comprehensive machineries for protein trafficking and proper function, maladies may still arise. Indeed, a growing body of evidence supports the view that peroxisomes are key players in the etiology and progression of oxidative stress-related disorders [23]. Environmental factors that interfere with peroxisome biogenesis may compound such effects (for more details, see 5.).

4.2.2. Peroxisome dysfunction and changes in the epigenetic landscape

4.2.2.1. DNA and histone (de)methylation

As peroxisomes have the potential to directly or indirectly alter the levels of a number of metabolites that function as substrates for DNMTs, HMTs, and HDMs, changes (or defects) in peroxisome function can be expected to influence the activities of these chromatin-modifying enzymes. However, so far, this view is only supported by two pieces of indirect evidence.

First, it has been shown that recurrent mutations in IDH1, a cytosolic and peroxisomal enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-KG (thereby reducing $NADP^+$ to NADPH) [36], are associated with a hypermethylation phenotype in hematologic (e.g., acute myeloid leukemia) and solid (e.g., glioma, chondrosarcoma, and cholangiocarcinoma) tumor malignancies (see [37], and references therein). Interestingly, these mutations occur at a single amino acid residue (R132) within the active site of IDH1, and result in a protein that can no longer exert its wild-type activity

but rather has acquired the ability to convert α-KG to the oncometabolite 2-hydroxyglutarate, thereby using NADPH as a cofactor. As 2-hydroxyglutarate functions as a competitive inhibitor of α-KG-dependent dioxygenases (e.g., JmjC-HDMs and TET 5mC hydroxylases) [38], this results in enhanced trimethylation of H3K9 and hypermethylation of CpG promoter regions [39,40]. Although the precise contribution of mutant peroxisomal IDH1 to cancer cell 2-hydroxyglutarate production remains to be established, it is quite likely that this pool of protein has the potential to affect the activity of chromatin-modifying enzymes (and hence the methylation status of chromatin). Relatedly, cartilage-specific $IDH1_{R132Q}$ knock-in mice and mice lacking PEX7, share a remarkable number of similar features, such as skeletal abnormalities, dwarfism, hypotonia, and high risk of death on the day of birth or prior to weaning [41].

Second, there is currently sufficient empirical evidence to argue that disturbances in peroxisome function affect cellular GSH levels (see [5], and references therein). As alterations in GSH homeostasis directly impact the glutathionylation state of histones as well as the *de novo* synthesis and availability of the universal methyl donor SAM (see 3.3.), it is reasonable to assume that changes in peroxisome activity may also influence DNA and histone methylation patterns. One argument in favor of this hypothesis is that polymorphisms in methionine metabolism genes have been described as possible disease modifiers in X-linked adrenoleukodystrophy (X-ALD) [42], a severe neurodegenerative disorder that is caused by defects in the peroxisomal ATP-binding cassette transporter protein ABCD1 and characterized by, among other things, the accumulation of very-long-chain fatty acids (VLCFAs), oxidative stress, bioenergetic failure, and axonal degeneration (see [43], and references therein). However, do note that despite the fact that loss of ABCD1 function clearly influences gene expression in cultured dermal fibroblasts and induced pluripotent stem cells, the global DNA methylation profiles of X-ALD cells is apparently indistinguishable from that of control cells [44]. A second argument is that disturbances in peroxisomal redox metabolism have an immediate impact on mitochondrial ROS production (see [5], and references therein), an event that can be directly linked to a decrease in mitochondrial DNA methylation [45].

4.2.2.2. Histone (de)acetylation

The enzymatic activities of HATs and HDACs, two classes of enzymes that control the acetylation state of histones, are tightly controlled by the nutritional, energetic, and redox state of a cell (see 2.2.2.). As many of these conditions are directly or indirectly influenced by changes in peroxisome activity (see 4.1.), it may not come as a surprise that malfunctioning of peroxisomes can be linked to changes in histone acetylation. For example, it has been shown that phytanic acid, a dietary fatty acid that is degraded via peroxisomal α-oxidation and best known for its accumulation in Refsum disease [3], enhances HDAC activity *in vitro* and reduces histone acetylation in Neuro2a cells, thereby inducing cell death [46]. In addition, it has been reported that sirtuin 1 (SIRT1), an NAD^+-dependent protein deacetylase that links transcriptional regulation directly to intracellular energetics, is dysregulated in the spinal cords of 12-month-old *Abcd1*$^{-/-}$ mice as well as in the affected white matter of X-ALD patients [43]. Finally, as the acetyl-moieties resulting from peroxisomal β-oxidation can leave the organelle [25,35], it is very likely that peroxisomal metabolism can also influence HAT activity. This is perhaps best illustrated by the observation that peroxisome-derived acetyl-moieties can serve as substrate for acetyl-CoA carboxylase [25], a cytosolic enzyme that regulates global histone acetylation by controlling the availability of acetyl-CoA for HATs [47].

Disturbances in peroxisomal metabolism may also result in a decrease of the intracellular acetyl-CoA and free CoA concentrations due to CoA sequestration (e.g., upon exposure to xenobiotics and drugs [5]).

4.2.2.3. Regulatory non-coding RNAs

As peroxisomes play a central role in cellular redox metabolism [5], and Dicer, a cytoplasmic ribonuclease III that processes pre-miRs to mature miRs, functions as an oxidative stress-responsive protein [7] - it is very likely that changes in peroxisome function also influence the biogenesis and processing of miRs. Experimental evidence that this may actually be the case has recently emerged. For example, it has been shown that enhanced catalase activity can attenuate the H_2O_2-induced upregulation of miR-153 in SH-SY5Y neuroblastoma cells [48]. MiR-153 targets, among others, the redox-sensitive transcription factor nuclear factor-erythroid 2-related factor 2 (NFE2L2), a master regulator of antioxidant transcriptional responses [48]. In addition, it has been demonstrated that the expression of several miRs is upregulated in PEX16-deficient human skin fibroblasts, and that peroxisomal dysfunction stimulates apoptotic cell death of articular chondrocytes and cartilage degradation in osteoarthritis patients and mice with type 2 diabetes through upregulation of miR-223 [49]. Finally, changes in Dicer activity also affect peroxisome function; this is perhaps best illustrated by the observation that inactivation of Dicer in mouse liver results in the upregulation of peroxisome proliferator-activated receptor (PPAR) alpha target proteins (e.g., peroxisomal β-oxidation enzymes) [50].

5. Peroxisomes and the cellular microenvironment

Peroxisomes are remarkably versatile and dynamic cell organelles whose size, shape, number, and protein content can vary greatly depending on cell type, developmental state, and environment [51]. Here we elaborate on microenvironmental factors that can modulate peroxisome biogenesis and function (Fig. 2). Gaining a better understanding of this relationship is a prerequisite for designing safe and effective therapeutic interventions (see 6.).

5.1. Nutrient availability and xenobiotic stimuli

It is well documented that cells can quickly adapt peroxisome number and function in response to changes in nutrient conditions and xenobiotic stimuli. Many of these adaptations are mediated through activation of PPAR-dependent signaling pathways. PPARs are a group of transcription factors (PPARα, PPARγ, and PPARδ) that constitute a subfamily of the nuclear receptor superfamily and function as lipid sensors and regulators of (lipid) metabolism (see [4], and references therein). All PPAR isoforms function as obligate heterodimers with retinoid X receptor (RXR), a nuclear receptor that is activated by 9-cis-retinoic acid, to regulate downstream target gene expression upon activation by their agonists. Important classes of PPAR agonists include, among others, naturally occurring fatty acids, fibrates, and thiazolidinediones. PPARs show distinct tissue distribution patterns and have pleiotropic effects on a wide range of cellular processes, including peroxisome biogenesis. For example, fatty acid and fibrate activation of PPARα stimulates peroxisome formation (e.g., through enhanced expression of PEX11α, a protein involved in peroxisome proliferation) and fatty acid β-oxidation in various tissues [51]. Also, treatment of murine 3T3-L1 pre-adipocyte cells with rosiglitazone, a potent thiazolidinedione insulin sensitizer, induces the expression of several *PEX* genes through activation of PPARγ [4]; and high fat

feeding increases PPARγ expression and the proliferation of peroxisomes in the hypothalamus [52]. Finally, a cardiomyocyte-specific loss of PPARδ in mice results in reduced expression of peroxisomal and mitochondrial β-oxidation genes and leads to cardiomyopathy [53]. Note that the extent to which these and other PPAR-mediated processes are influenced is not only species-dependent [51], but can also be affected by the availability of specific transcriptional coactivators, such as PPARGC1A [54].

The complex interplay between peroxisomes, PPARs, and epigenetic regulation mechanisms is further strengthened by additional important observations. For example, it has been demonstrated that butyrate-producing probiotics (e.g. 4-phenylbutyrate) can upregulate peroxisome activity through inactivation of HDAC activity (see 2.2.2.), an event that enhances the expression of PPARα [55]. Also, it has been reported that PPAR levels can be controlled by miRs whose expression levels are affected by oxygen tension [56] or inflammatory stimuli [57]. Regarding the latter, it is important to note that (i) peroxisomal β-oxidation is also involved in the breakdown of eicosanoids, a class of bioactive lipid mediators that can elicit a broad range of inflammatory reactions [3], and (ii) impairment of peroxisome function can contribute significantly to the prolongation and intensification of inflammatory reactions [23]. These and other findings have led to the idea that even mild peroxisome biogenesis defects (e.g., caused by a deficiency in PEX11α) may lead to an accumulation (e.g., VLCFAs, eicosanoids) and/or depletion (e.g., polyunsaturated fatty acids, alkyl ether-phospholipids) of PPAR ligands, thereby deregulating PPAR activity and peroxisome/PPAR-feedback loops [58].

5.2. Oxidative stress

Over the years, it has become evident that alterations in the cellular redox balance can influence peroxisome biogenesis and function at various levels. In the following paragraphs, we provide a set of examples of how oxidative stress may regulate these processes at the transcriptional, post-transcriptional, and post-translational level. Note that most of these examples focus on the expression, subcellular localization, and activity of catalase, the best characterized peroxisomal antioxidant enzyme.

First, it has been reported that catalase is downregulated in many patient and mouse tumor tissues, and that this phenomenon can be mimicked *in cellulo* by exposing hepatocellular carcinoma cells to ROS [59,60]. A molecular analysis of these cells revealed that ROS treatment resulted in epigenetic silencing of catalase through hypermethylation of CpG island II on the catalase promoter [59] and methylation of CpG island in the *POU2F1* promoter [60]. Note that POU2F1 is a transcription factor that binds to the promoter of catalase to upregulate its expression.

Second, catalase activity can also be regulated at the post-transcriptional level via miR expression. For example, sublethal doses of H_2O_2 have been shown to upregulate the expression of miR-30b, a small ncRNA that inhibits the expression of catalase in human retinal pigment epithelial cells [61]. In addition, it has been reported that receptor-interacting protein 1, a key mediator in cell survival and death signaling, blunts the anticancer activity of cisplatin by decreasing the expression of miR-146a, another small ncRNA that represses catalase expression [62].

Lastly, catalase activity can also be influenced at the post-translational level by S-nitrosylation and oxidative stress-induced changes in subcellular localization [5]. How S-nitrosylation influences catalase activity remains to be investigated. Regarding the observation that oxidative stress can affect catalase localization, it is important to point

out that peroxisome biogenesis and function become gradually impaired as cells age [63]. Indeed, Terlecky and co-workers have shown that cellular aging, a process associated with an increase in oxidative stress, is accompanied with (i) a decrease in the import of proteins containing a C-terminal peroxisomal targeting signal (PTS1), (ii) an accumulation of PEX5, the shuttling import receptor for PTS1 proteins, on the peroxisomal membrane, and (iii) an increase in peroxisome number. These phenomena may very well be explained by the fact that PEX5 functions as a redox-sensitive protein, a property that can be attributed to an evolutionarily conserved cysteine residue (e.g., Cys11 in human PEX5) that is involved in receptor monoubiquitination [64]. As this monoubiquitination event marks PEX5 for recycling [64] and can potentially also serve as a quality control mechanism to eliminate peroxisomes with a defective protein import machinery [65], the increased redox balance of the cytosol in aging cells can be expected to impair matrix protein import and, possibly also, peroxisome degradation pathways. Interestingly, as (i) the extent of mislocalization of individual PTS1 proteins apparently depends on the strength of the targeting signal, and (ii) catalase, in contrast to most peroxisomal H_2O_2-producing oxidases, contains a relatively weak PTS1, the existence of a self-perpetuating negative protein import spiral causing oxidative stress and peroxisome dysfunction has been postulated [66]. However, as the monoubiquitinable cysteine residue in the N-terminus of PEX5 and the weak PTS1 of catalase are highly conserved in virtually all mammalian species, these features may also have evolved to allow cells to rapidly respond to oxidative insults in the cytosol [64]. The latter idea is supported by the observation that valosin-containing protein, an ATPase associated with diverse cellular activities, can sense and regulate H_2O_2 levels in the cytosol by affecting the retention time of (newly-synthesized) catalase within this cellular compartment [67].

5.3. Oxygen tension

Oxygen is a key intermediate and critical signaling molecule for multiple cellular processes. As changes in oxygen concentration can contribute to cell dysfunction, cells have evolved mechanisms to rapidly sense and respond to changes in oxygen tension in their microenvironment. One such mechanism involves the action of hypoxia-inducible factors (HIFs), a class of transcriptional regulators of genes that mediate cellular adaptation to hypoxia [68].

As peroxisomal flavin oxidases consume molecular oxygen (see 4.1.1.), it can be expected that changes in oxygen tension also affect peroxisome function. That this is indeed the case is supported by findings in which peroxisome volume and catalase activity are two- and four-fold increased, respectively, in hyperoxia (99% O_2, 1% CO_2)-adapted Chinese hamster ovary cells, as compared to parental cells that are cultivated at normoxic conditions (20% O_2, 1% CO_2, 79% N_2) [69]. In addition, it has been reported that, under hypoxic conditions (<2% O_2, 5% CO_2, balanced N_2), peroxisome number and function are upregulated in human glioblastoma cells [70] but strongly reduced in human skin fibroblasts [71]. Note that the increase in peroxisome activity in hypoxic glioblastoma cells has been linked to enhanced expression and increased nuclear localization of PPARα [70]. Finally, it has recently been demonstrated that EPAS1 (HIF2A) functions as a negative regulator of peroxisome abundance and metabolism in mouse hepatocytes and human clear cell renal cell carcinomas [72]. This observation led these authors to hypothesize that the hypoxia-driven expression of EPAS1 may allow cells to adapt their peroxisomal activities to the availability of oxygen.

5.4. Temperature

More than a decade ago, it was reported that culturing fibroblast cell lines from PBD patients with an intermediate (e.g., NALD) or milder (e.g., IRD) phenotype (see 4.2.1.) at lower temperature frequently resulted in the rescue of peroxisome biogenesis errors and functional defects [73]. This finding suggested that mutations in the respective *PEX* genes (e.g., *PEX1*, *PEX2*, *PEX5*, *PEX6*, or *PEX13*) yield structurally unstable proteins, hinting that chemical or pharmacological chaperones may restore biological function and serve as a strategy for therapeutic treatment (see 6.5.). In addition, these outcomes suggested that precautions to prevent fever may be necessary as part of treatment regimen of PBD patients with temperature-sensitive mutations [73]. Finally, with an eye toward predictive analyses, an examination of the temperature sensitivity of peroxisome biogenesis in fibroblasts of newborns may help to assess the severity of, and identify a prognosis for, patients with peroxisome disease [73].

6. Therapeutic Interventions

Peroxisomal disorders comprise a family of genetically heterogeneous and progressive diseases that display a broad phenotypic spectrum [7,35]. At present, effective treatments are still limited and not universally applicable due to the complex nature of these disorders. For example, in patients with severe ZSD, treatment is mainly symptomatic and supportive [7]. Fortunately, most patients with milder symptoms have defects that retain residual protein function, raising the possibility that specific treatments may enhance protein activity (e.g., through upregulation or stabilization of the affected protein) or mitigate downstream metabolic perturbations (e.g., the lack of plasmalogens, the accumulation of VLCFAs, and mitochondrial dysfunction). In the following sections, we discuss therapies that have already been explored or are currently in development to treat patients suffering from peroxisomal disorders (Fig. 3). Note that we will only focus on treatments that act directly at the level of the epigenome, and not on surgical procedures (e.g., haematopoietic stem cell or bone marrow transplantation) that have already proven to halt disease progression (e.g., in early-stage cerebral ALD) [74].

6.1. Diet therapies

Strategies to mitigate the symptoms of peroxisomal disorders often include dietary changes. These can include supplementation of missing metabolites (e.g., primary bile acids, docosahexaenoic acid (DHA), and plasmalogen precursors), dietary restrictions of metabolites that accumulate (e.g., phytanic acid), or diet treatments that normalize biochemical parameters (e.g., VLCFA levels).

6.1.1. Lorenzo's oil

The perhaps best-known diet therapy for peroxisomal disorders is "Lorenzo's oil", a 4:1 mixture of glyceryl trioleate and glyceryl trierucate. In combination with a moderate reduction of fat in the diet, this therapy has been shown to significantly lower the levels of VLCFAs in plasma of X-ALD and ZS patients (see [74], and references therein). Currently, this process is thought to occur through inhibition of ELOVL1, a fatty acid elongase responsible for the synthesis of saturated and monounsaturated VLCFAs [75]. Unfortunately, Lorenzo's oil appears to be ineffective in halting or preventing the neurological decline in cerebral variants of X-ALD [74]. However, a combination therapy of Lorenzo's oil, DHA (see below), and medium-chain triglyceride milk can

apparently alleviate liver damage, muscle weakness, and neurological deficits in a ZS patient [76].

6.1.2. DHA

Interestingly, a clinical study evaluating the efficacy of DHA in ZSD patients demonstrated that supplementation of DHA ethyl ester restored blood DHA values within a few weeks [77]. In addition, this work showed that such treatment also normalized various biochemical (e.g., VLCFAs and plasmalogen levels, brain myelin) and clinical (e.g., vision, liver function, muscle tone, social contact) parameters in several patients. ZS model mice, when treated with DHA, do not show overall improvements, despite the normalization of DHA levels in the brain [78].

6.1.3. Plasmalogen precursors

One group of peroxisomal disorders, which include the RCDP patients, is characterized by a failure in plasmalogen biosynthesis [79]. As peroxisomes are only necessary for the initial biosynthesis steps of this unique class of molecules, it should, in principle, be possible to recover plasmalogen levels in RCDP (and ZSD) patients by oral supplementation of plasmalogen precursors (e.g., 1-0-octadecyl-sn-glycerol) that can enter the plasmalogen biosynthetic pathway downstream of the peroxisomal steps. This idea is supported by the observations that 1-0-octadecyl-sn-glycerol supplementation significantly increased plasmalogen levels in erythrocytes from ZSD patients (see [79], and references therein) as well as in erythrocytes and multiple peripheral organs (e.g., liver, kidney, heart, lung, testis, and the eye) of PEX7-deficient mice [80]. Interestingly, when administered prior to the realization of major pathological changes, plasmalogen replacement therapy also halted or slowed the development of pathology normally observed in Pex7 knockout mice (e.g., testicular degeneration and cataract formation) [80].Unfortunately, the 1-0-octadecyl-sn-glycerol-containing diet could only marginally increase plasmalogen levels in nervous tissues; how this diet affects the lifespan of these mice is unknown at present [80].

6.1.4. Primary bile acids

The final steps of bile acid synthesis occur in peroxisomes, and defects in peroxisome assembly have been shown to lead to an increase of C27, and a decrease of C24 bile acids [3]. Studies in *Pex2*$^{-/-}$ ZS mice have demonstrated that feeding these mice a solution containing cholic acid and ursodeoxycholic acid improved bile acid deficiency in bile and liver [81]. In addition, although mitochondrial alterations persisted, this treatment prolonged postnatal survival. In the meantime, the United States Food and Drug Administration has recently approved cholic acid capsules (Cholbam) to treat adults and children with bile acid synthesis disorders, including ZSDs; this is the first and only drug of its kind (see http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm438572.htm).

6.2. Peroxisome proliferators

Peroxisome proliferators are a group of structurally diverse molecules that function as agonists of PPARs (see 5.1.), thereby affecting a large network of cellular activities, ranging from (peroxisomal) metabolism and inflammation, to cellular differentiation and development [4,82]. Common pharmaceutical applications include fibrates (e.g., fenofibrate and bezafibrate) and thiazolidinediones (e.g., rosiglitazone and pioglitazone) - established therapeutics for hypercholesterolemia and type 2 diabetes,

respectively. While these drugs may not be beneficial for ZSD patients where the organelle is completely absent, they may be of value in more modest forms of peroxisome disease. For instance, there is much evidence to argue that fibrates can restore VLCFA β-oxidation in X-ALD fibroblasts as well as in the liver of *Abcd1*$^{-/-}$ mice (see [83], and references therein). However, the mechanisms underlying this phenomenon appear to be complex and diverse (see continued discussion in next paragraph). In addition, there is some evidence that PPARγ agonists such as rosiglitazone and pioglitazone can protect hypothalamic pro-opiomelanocortin neurons [52] and cortical neurons [84] from inflammatory mediators via an improvement in peroxisome function.

To understand how fibrates may lower VLCFA levels in various cell types and tissues, it is essential to know that (i) mammalian peroxisomes contain three ABCD proteins (ABCD1/ALDP, ABCD2/ALDPR, and ABCD3/PMP70), each displaying substantial overlap in function; (ii) overexpression of ABCD2 or ABCD3 can rescue peroxisomal β-oxidation in X-ALD fibroblasts; and (iii) fibrate therapy stimulates ABCD2 and ABCD3 expression in a PPARα-dependent manner in rodent liver and adrenal glands, but not in brain and testis (see [74], and references therein). Despite the latter observation, a detailed analysis of the promoter region of the *ABCD2* gene failed to identify functional peroxisome proliferator response elements, suggesting that the PPARα-dependent activation of this gene may occur through an indirect mechanism [74]. Evidence in favor of such a conclusion was provided by experiments showing that the mouse and human *ABCD2* promoters contain DNA sequences that are recognized by sterol regulatory element (SRE)-binding proteins (SREBPs), a group of transcription factors whose expression depends on PPARα activity [74]. Importantly, CoA esters of different fibrates (e.g., bezafibrate and gemfibrozil) have also been shown to reduce VLCFA accumulation in X-ALD fibroblasts by directly inhibiting the fatty acid elongase activity of ELOVL1 [83,85].

As PPARs are involved in a wide array of metabolic processes, it is logical that treatment with these compounds could cause many undesirable side effects. PPAR agonists such as clofibrate and fenofibrate have been approved for use in humans and are generally considered safe, however there is a concern that the molecules may be acting as nongenotoxic carcinogens – a circumstance certainly true in rodents.

6.3. HDAC activity modulators

HDACs are established and validated targets for the treatment of many diseases, ranging from metabolic and inflammatory disorders to neurodegeneration and cancer. A growing body of evidence has accumulated that suggests patients with peroxisomal defects, especially those with defects in ABCD1, may benefit from treatment with agents that modulate HDAC activity. Promising molecules include, among others, the class I and II HDAC inhibitors 4-phenylbutyrate (4-PBA) and suberoylanilide hydroxamic acid (SAHA), and the putative class III HDAC activator resveratrol.

4-PBA, an FDA-approved HDAC inhibitor for the treatment of urea cycle disorders, has been shown to significantly increase the number of peroxisomes, the expression of ABCD2, and VLCFA β-oxidation in cells from X-ALD patients and *Abcd1*$^{-/-}$ mice (see [74], and references therein). In addition, this compound upregulates the transcription of ABCD2 and PEX11α in fibroblasts from ZSD patients, to restore peroxisomal β-oxidation and plasmalogen content in cells from NALD and IRD (but not ZS) patients, and to increase the number of peroxisomes and the expression of peroxisomal genes

(e.g., ABCD2) in rat liver as well as in primary rat hepatocytes and glial cell cultures (see [74], and references therein). Interestingly, the latter study also demonstrated that the dose-dependent increase in ABCD2 expression required the recruitment of HDAC1, but not PPARα, to the *ABCD2* promoter. Note that 4-PBE also has documented chemical chaperone activity (see 6.5.).

SAHA, an FDA-approved HDAC inhibitor for the treatment of cutaneous T-cell lymphoma, has been shown to lower VLCFAs in the central nervous system of *Abcd1*$^{-/-}$ mice, the target tissue in human X-ALD pathology [86]. In addition, in X-ALD fibroblasts, the compound increases the expression levels of ABCD2 and ABCD3, normalizes peroxisomal β-oxidation, and downregulates the expression of ELOVL1 [86]. Interestingly, a recent study reported that SAHA attenuates mitochondrial dysfunction and energetic failure in *ABCD1*-silenced human U87 astrocytes and rat B12 oligodendrocytes through PPARGC1A- and PPARGC1B-induced mitochondrial biogenesis [87]. These findings also reinforce the view that intact peroxisomes are essential to maintain mitochondrial integrity and function.

Resveratrol is a dietary phytophenol that exerts its biological activity via a number of mechanisms that may vary depending on cell type, dose, and dosing schedule [88]. Cellular processes shown to be affected by this compound include, among others, metabolism, inflammation, cell signaling, and posttranslational modification. However, resveratrol's pharmacological mode of action is not yet fully understood, and many potential targets (e.g., histone deacetylase inhibition, AMP-activated protein kinase activation, and miRNA modulation) have been proposed [88]. It was recently demonstrated that oral administration of resveratrol could normalize redox homeostasis, mitochondrial respiration, bioenergetic failure, and axonal degradation and associated locomotor disabilities in *Abcd1*$^{-/-}$ mice [43]. In addition, as (i) SIRT1 activity was impaired in the spinal cord of *Abcd1*$^{-/-}$ mice as well as in the affected white matter of X-ALD patients, and (ii) a moderate transgenic overexpression of SIRT1 in *Abcd1*$^{-/-}$ mice yielded similar beneficial effects, the authors concluded that the resveratrol-dependent health benefits are mediated via activation of SIRT1. Whether resveratrol activates SIRT1 directly or indirectly, remains to be investigated. In this context, it is interesting to note that this compound has been shown to directly inhibit cAMP-specific phosphodiesterases [89]. This in turn triggers the activation of AMP-activated protein kinase, a metabolic sensor that enhances SIRT1 activity by increasing the intracellular NAD^+ levels. In addition, resveratrol may also influence the expression of various miRs [90].

In summary, these findings suggest that HDAC activity modulators such as 4-PBA and SAHA are potential therapeutics for treatment of X-ALD. However, whether or not these compounds will be of any benefit in the context of peroxisomal disorders is unclear. This is perhaps best illustrated by the fact that, despite 4-PBA's ability to upregulate peroxisome number and VLCFA β-oxidation in various cell types and tissues (see above), a trial in adrenomyeloneuropathy patients failed to demonstrate any clinical efficacy [74].

6.4. Antioxidants

Currently, there is ample evidence that peroxisome biogenesis, peroxisome function, and maintenance of cellular redox balance are intricately connected processes [23]. This is nicely illustrated by the observations that peroxisome biogenesis and function decline during cellular aging, a process associated with oxidative stress, and that catalase-SKL

(a catalase derivative with enhanced peroxisome targeting) and N-acetylcysteine (a thiol-reducing agent and precursor of GSH) can restore peroxisomal protein import and normal oxidative state in aging cells [63,64,91]. Interestingly, N-acetylcysteine has also been shown (i) to attenuate peroxisome dysfunction (e.g., β-oxidation, plasmalogen biosynthesis) and oxidative stress in a PPARα-dependent manner in fetal mouse brain upon exposure of the mothers to lipopolysaccharides, a major constituent of the outer membrane of Gram-negative bacteria and primary inducer of chronic inflammatory diseases and septic shock [92], and (ii) to scavenge VLCFA-dependent ROS generation in human X-ALD fibroblasts [93]. Importantly, the latter study also demonstrated that the oral administration of an antioxidant cocktail consisting of N-acetylcysteine, lipoic acid, and Trolox (a water-soluble analog of vitamin E) could block oxidative damage to proteins and DNA in the spinal cord from *Abcd1*$^{-/-}$ mice and prevent and arrest progression of locomotor deficits in *Abcd1*$^{-/-}$/*Abcd2*$^{-/-}$ mice. Taken together, these findings open new perspectives for the use of antioxidant cocktails for patient treatment. A clinical trial on the use of antioxidants to slow progression of adrenomyeloneuropathy (NCT01495260) has recently been completed but, to date, the results obtained have not been published.

6.5. Chemical chaperones

A relatively unstudied intervention includes the use of chemical chaperones, a group of small osmolytes (e.g., betaine, glycerol, trimethylamine N-oxide, L-proline, L-arginine) and hydrophobic compounds (e.g., 4-PBA, bile acids) that assist in the proper folding of proteins: osmolytes increase the stability of proteins without affecting their activity through sequestration of water molecules, an event that increases the free energy of the protein's unfolded state more than its folded state; and hydrophobic compounds prevent protein aggregation by shielding exposed hydrophobic segments of unfolded proteins [94]. The chemical chaperones trimethylamine N-oxide, glycerol, betaine, and L-proline have been shown to partially recover matrix protein import in primary fibroblasts from a mildly-affected ZSD patient containing the common disease allele PEX1-p.Gly843Asp (G843D), a mutation that causes a conformational change and renders the protein inactive [95]. Another study reported that supplementation of L-arginine to the cell culture medium can improve peroxisome biogenesis and function in human fibroblasts with mild missense mutations in *PEX1*, *PEX6*, and *PEX12* [96]. Betaine is also currently being evaluated in a clinical trial (NCT01838941) that is intended to test whether or not it can restore key peroxisome functions (e.g., VLCFA and bile acid profiles, plasmalogen levels) in the blood of PEX1-G843D patients.

6.6. Nonsense suppressors

Nonsense suppressors enable tRNA to bypass stop-codons and continue translation. Popular examples include the aminoglycoside antibiotics gentamicin and geneticin (G418). A few years ago, it was reported that addition of G418 to the cell culture medium could partially rescue peroxisome biogenesis and significantly improve peroxisomal lipid metabolism in ZSD skin fibroblasts from patients with stable PEX2 or PEX12 nonsense transcripts [97]. These findings support the idea that nonsense suppressor therapies may be beneficial for a subset of ZSD patients.

6.7. Enzyme replacement therapy

Enzyme replacement therapy (ERT) is a potentially attractive medical treatment approach in which proteins are administered to patients to compensate for the loss of a

particular enzyme that is dysfunctional or absent. Over the years, ERT has been successfully employed in such systemic diseases as hemophilia and lysosomal storage disorders, in which intravenously administered enzymes can easily reach therapeutic levels in relevant target organs [98]. However, development and characterization of short peptides that promote the cellular uptake of specific cargo molecules, have broadened the spectrum of disorders that are potentially targets for ERT. Catalase-SKL is one such cell penetrating peptide-containing protein biologic; importantly, it has been shown to protect primary rat cortical/hippocampal neurons from amyloid-β-induced toxicity [99] and to correct oxidative stress-induced pathophysiology in incipient diabetic retinopathy [100]. As patients with peroxisomal diseases experience neurodegeneration [7], these studies not only reinforce the idea that antioxidants can be part of effective therapeutic strategies (see 6.4.), but also underscore the powerful potential of ERT in dealing with ZSDs and PEDs.

7. Conclusions and perspectives

Peroxisomes are linked to the epigenetic landscape in ways only beginning to be understood. A plethora of compelling evidence points to the importance of such bidirectional crosstalk between the systems, but a clear mechanistic understanding of this cellular relationship remains elusive. Contributing to the complexity are the following: (i) the activity of many enzymes involved in epigenetic regulation is subject to exquisite regulation by metabolite concentrations (see 3.); (ii) the intracellular levels of many of these metabolites are controlled by peroxisomal as well as mitochondrial metabolism (see 4.1.); (iii) the cause and effect relationships between peroxisomal (dys)function and epigenetic alterations are clearly modulated by a number of complex feedforward/feedback mechanisms (see 5.); and (iv) the cellular epigenetic landscape and its bidirectional relationship with peroxisomes and associated organelle function can differ significantly among cell types, tissues, organs, organisms, and disease states. These complexities are relevant to the development of therapeutic strategies designed to thwart peroxisomal disease; what is effective in one patient, may not be in another. However, the new information outlined in this review, including the only recently described revelation that peroxisomes are highly druggable targets, provides hope that a door has opened for unparalleled rapid advancement in the development of compounds and strategies to combat the devastating circumstance that is human peroxisomal disease.

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Figure legends

Fig. 1. Peroxisomal metabolites have the potential to influence the epigenetic landscape. Molecules that can be linked to peroxisomal metabolism are shown in blue. The arrows and the bars represent stimulatory and inhibitory signals, respectively. Multiple arrows represent a series of enzymatic steps not described in the text. α-KG, α-ketoglutarate; acetyl-CoA, acetyl-coenzyme A; DDM, DNA demethylase; DNMT, DNA methyltransferase; FAD(H_2), (reduced) flavin adenine dinucleotide; GR, glutathione reductase; GSH, (reduced) glutathione; GSSG, oxidized glutathione; HAT, histone acetylase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; NAD(P)(H), (reduced) nicotinamide adenine dinucleotide (phosphate); ncRNA, non-coding RNA; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

Fig. 2. Influence of genetic and environmental factors on peroxisome biogenesis and function.

Fig. 3. Potential epigenetic therapies to treat peroxisomal disease. 4-PBA, 4-phenylbutyrate; BA, bile acid; CC, chemical chaperone; DHA, docosahexaenoic acid; ERT, enzyme replacement therapy; HDAC, histone deacetylase; NAC, N-acetylcysteine; NS, nonsense; PP, plasmalogen precursor; SAHA, suberoylanilide hydroxamic acid.